

iGEM TU/e 2016

Biomedical Engineering

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Small Culturing

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1 Small Culturing

Estimated bench time: 30 minutes

Estimated total time: 17 hours

Purpose: Amplification of the bacteria with the inserted plasmid.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

1.1 Materials

- Antibiotic stock
- Bunsen Burner
- LB-agar plates with bacteria
- LB Medium
- Pipetboy and pipettes
- Pipettes and tips
- Shake incubator
- Sterile culture tubes

1.2 Setup & Protocol

- Fill sterile culture tubes with 8 mL of LB medium (or 25 mL when making small culture after transformation into BL21).
- Add 8 μ L antibiotic stock (kanamycin or chloramphenicol) to the sterile culture tubes to get the correct working concentration (or 25 μ L when making small culture after transformation into BL21).
- Pick bacterial colonies near the Bunsen flame with a pipette tip (another option is to continue with the picked bacteria which are verified by colony PCR).
- Load the colonies into correct labeled sterile culture tubes.
- Pipette up and down such that they are mixed well.
- Close the culture tubes and place them into the shake incubator.
- Grow the bacteria overnight at 37 °C on 250 rpm.

2 (Optional) Glycerol Stock

Estimated bench time: 5 minutes per sample

Estimated total time: 10 minutes per sample

Purpose: Preparing the bacteria for long term storage in the -80 °C freezer.

For more information, check out the 'General Necessities protocol'.

3 (Optional) Miniprep

Estimated bench time: 1 hour

Estimated total time: 1 hour

Purpose: Plasmid extraction from the bacteria.

For more information, check out the general 'Miniprep protocol'.